

## ABSTRACT

Shell disease has recently been recognized as a common problem in blue crabs in certain areas of the Albemarle-Pamlico Estuary. This disease is a common problem in crustacean (crab, shrimp, lobster) populations and is most commonly associated with stressful conditions, such as polluted ecosystems or adverse aquaculture environments. While a number of pathogens, especially bacteria, have been isolated from shell disease lesions, the mechanisms leading to development of this disease are unclear. The difficulty in reproducing the disease by simply challenging healthy crustaceans with the putative causative bacteria suggests that host immunity may play a very important role in disease development.

Our studies have shown that shell disease lesions from blue crabs in the A/P Estuary have large numbers of bacteria; all of these bacteria have enzymes, such as chitinase and lipase, that are believed to be pathogenic markers that allow these bacteria to degrade the blue crab shell. However, the shell of clinically normal crabs that do not have shell disease also have large numbers of bacteria. These bacteria have the same enzymatic activities as those cultured from crabs with shell disease. This evidence supports the hypothesis that a change in host defenses may be pivotal to defending against these endogenous pathogens.

We have found that the blood (hemolymph) of blue crabs has a potent activity that kills many of the bacteria that are present on both clinically normal crabs and those having shell disease. Our demonstration of activity in shell extracts also suggests a primary defensive role in this activity in protecting against shell disease. This antibacterial activity is bactericidal (i.e., it kills these bacteria). Our studies have also demonstrated that this activity is sensitive to high temperatures and is inhibited by sodium chloride. The activity is also stable after multiple freeze-thaw cycles and is very stable during storage at  $-70^{\circ}\text{C}$ . The latter properties make this activity a potentially useful substance for field monitoring, as described below.

We developed a sensitive, reproducible and quantitative assay for measuring this antibacterial activity in individual blue crabs. This modified turbidometric assay has allowed us to test activity using very small samples. This test demonstrated that there were statistically significant differences in the antibacterial activity of different groups of crabs. First, there was a significantly lower antibacterial activity in crabs with shell disease compared to clinically normal crabs collected from the same geographic area. Second, there was a significantly lower activity in blue crabs collected from riverine areas of the A/P Estuary (e.g., Pamlico River, Pungo River) compared to those